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Effect of Habitat Locations on the Bacteriological and Physicochemical Assessment of Aquaculture Freshwater Catfish (*Clarias gariepinus*) Using Small Scale Depuration System

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Authors' contributions

This work was carried out in collaboration between all authors. Author ACA designed the study, performed the statistical analysis and managed the literature searches. Author ECF wrote the protocol and wrote the first draft of the manuscript. Authors OES and ECC managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of the study was to investigate the effect of habitat locations on the bacteriological and physicochemical assessment of aquaculture freshwater fish (*Clarias gariepinus)* using a small scale depuration system. Catfish samples were harvested from two different locations, Michael Okpara University of Agriculture (MOUAU) and Umugbalu fish farm.

Methodology: The fish samples were subjected to depuration for a period of 48 h. The total bacteria count of the fish samples was determined and the isolates characterized before and after

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depuration time. The total bacteria count (TBC) and other selected pathogenic bacteria in water and the fish organs recorded significant difference ($p < 0.05$) with TBC having the highest (1.90 x 10^6cfuml^{-1}) in water sample from Umugbalu habitat. The TBC and other pathogenic bacteria from different fish organs (gill, muscle and gut) differed, which recorded 1.52 x 10^6, 1.41 x 10^6, 1.60 x 10^6cfug^{-1} (TBC); 9.9 x 10^5, 9.0 x 10^5, 9.5 x 10^5cfug^{-1} (*Coliform*); 2.0 x 10^5, <10^1, 1.5 x 10^5cfug^{-1} (*Listeria spp*); 3.5 x 10^5, 2.7 x 10^5, 4.0 x 10^6cfug^{-1} (*Salmonella spp*) respectively as typically observed in samples from MOUAU habitat. For pH, temperature, salinity and turbidity, water sample from MOUAU had the lowest values of 5.45, 30.0^{o}C, 4.10ppt and 20.0NTU and 7.60mg/l respectively.

Conclusion: Small scale depuration system was adequate for the assessment of bacterial quality of the water and the freshwater fish organs. The results obtained in this study have underscored the importance of adequate processing and cooking prior to consumption of freshwater fish. The Physicochemical parameters of the ecosystem investigated, differed from one location to the other.

Keywords: Depuration; catfish; habitat; fish organs.

1. INTRODUCTION

Fish contributes about 60% of the world supply of protein and 60% of the developing countries derives more than 30% of their animal protein from fish [1]. Fish consumed for protein improves nutrition due to its high biological value in terms of high protein retention in the body, low cholesterol level and presence of essential amino acids [2]. Fish are generally regarded as safe, nutritious and beneficial but aquaculture products have sometimes been associated with certain food safety issues [3].

Several studies have demonstrated that bacteria encountered in different fish are potentially pathogenic under certain conditions [4]. The affected fish produces fish diseases which cause economic losses not only from mortality but also treatment expenses to the final consumer [4]. Fish and shellfish not only transmit disease to man but to themselves which are subject to many diseases. They are capable of transmitting many of the established food borne microbial infection and intoxication. It has been observed that the speed at which a product spoils is also related to the initial microbial load on it; the higher the count, the sooner the spoilage occurs [1]. That is why the initial microbiology of fish skin, gills and gastrointestinal tract was subjected to many researches, as fishes take a large number of bacteria into their gut from water sediment and food [4]. The microbial safety of sea foods is related to their feeding habits and the quality of their ecosystems as well as their handling during marketing and retail operations [5]. Fishes are reared in different culture media or controlled environment which could be ponds (concrete or earthen), vats (wood or fiber) and plastic. Among these culture system, concrete and earthen ponds are widely used [6].

However, shellfish are being sold without any sanitary control, creating a public health risk [7] due to possible accumulation of pathogenic bacteria if the extraction and harvesting areas are contaminated by residual waters [8] or if they are handled without proper hygiene regulations [9]. Some of the most hazardous bacteria associated with the consumption of shellfish species include *Salmonella spp.*, *Escherichia coli*, *Listeria monocytogenes*, *Vibrio parahaemolyticus* and *V. cholera* [9,10]. The consumption of fresh African Catfish (*Clarias gariepinus*) is on the increase in both rural and urban centers in Nigeria [11], where most 'point and kill joints' (a restaurant where fish are kept alive, the customer chooses the desired size and it is killed and made into pepper soup as a delicacy) are present in our local restaurants and eatery houses, where they are sold as a delicacy. The improper processing of these fishes posses a lot of public health hazards to the consumers.

Since depuration is a process by which shellfish are held in tanks of clean seawater under conditions which maximize the natural filtering activity that results in expulsion of intestinal contents, it could enhance elimination of contaminants from the fish, and prevents their recontamination prior to sale [10]. These treatments have been of research interest to several workers [12] with emphasis on depuration of sea foods harvested from marine waters. In order to address such public health problems that are associated with catfish and tilapia consumption, the aim of this research is to

assess the bacteriological quality of freshwater fish (catfish) before and after depuration. Furthermore, the physicochemical and bacteriological qualities of their habitat (pond) locations were evaluated and it effect on the depuration of freshwater catfish. Results of the assessments will invariably address public health challenges associated with freshwater fish consumption.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Study site

The study was carried out in Ikwano local government in Abia state, which covered two freshwater ecosystem or aquaculture medium (concrete ponds) where freshwater Catfish (*Clarias gariepinus*) were reared. The two sites used in this study are Michael Okpara University of Agriculture Umudike Fish Farm and Eze Chiaghanti Okeiyi Fish Pond at Umugbalu village, both in Ikwano LGA of Abia state. The minimum distance between one location and the other is 2-3 km.

2.1.2 Water sampling

Water sample (500 ml) was collected in sterile conical flasks. The depth of water ranged from 0.8 m to 1.2 m. After on-site rinsing of the conical flasks, three (3) 500 ml samples from each site or pond were collected from 0.2 m above the bottom of the pond. Water samples were store in ice-pack cooler and transported to the laboratory within 1 h of collection for microbiological and physicochemical analyses.

2.1.3 Collection of fish sample

The fish species was identified at the Department of Fisheries and Aquatic Resource Management, Michael Okpara University of Agriculture Umudike, Abia state. Matured fish sizes with appropriate weight for human consumption was collected manually at each location, placed in plastic container of 50 litres capacity containing water from the location. The collected sample were transported to the Department of Food Science and Technology (Food Microbiology Laboratory), Michael Okpara University of Agriculture Umudike, within 30 min of collection for both microbiological analyses of the fresh water sample and depurated fish samples for 48 h.

2.1.4 Physico-chemical characteristics of water samples

The various physico-chemical parameters were analyzed according to the method of [13] and data were recorded during the study period. Physico-chemical characteristics of water samples were carried out in the Department of Environmental Management and Toxicology laboratory, Michael Okpara University of Agriculture Umudike, Abia State.

2.1.5 Depuration period

Catfish from both locations were depurated separately for 48 h and readings taken at 12 h intervals (0, 12, 24, 36, 48 h each). Samples were collected and microbiological analysis was carried out as described by [14].

2.2 Sample Preparation for Microbial Count Using Serial Dilution Technique

The fresh fishes were washed and clean with sterile distilled water and cotton wool, after which they were beheaded. Their gill, gut and muscle (meat) were carefully removed by fine dissection using sterile scalpel. Microbial analysis was carried out on the water and fish sample according to the method described by [14]. For the fish species and for the entire experiment 0.1 ml of appropriate dilution was used for inoculation on the molten. Tryptone soy agar and the selective media for the enumeration of *Coliforms*, *Listeria* and *Salmonella spp.* The microbial load for each water and fish samples were recorded in $cfuml^{-1}$ for water samples and $cfug^{-1}$ for fish samples. This experiment was carried out on the water samples (from different habitat locations), depuration medium and on the fish samples before (0 h) and after depuration at 12h intervals (12, 24, 36, 48 h) was recorded.

2.2.1 Enumeration of *Coliforms*

Sorbitol MacConkey agar (CM0813) was prepared according to manufacturer's instruction. Cefixime Tellurite selective supplement (SR0172E) was used for the isolation of *Coliform*. Pink colonies which were observed and counted with colony counter indicated the presence of Coliform.

2.2.2 Enumeration of *Listeria spp*

Brilliance *Listeria* agar base (CM1080) was prepared according to manufacturer's instruction.

One vial of Brilliance *Listeria* selective supplement (SR0227E) and Brilliance *Listeria* differential supplement (SR0228E) were added for the isolation of *Listeria spp*. Green colonies which indicated the presence of *Listeria spp* were observed and counted with colony counter.

2.2.3 Enumeration of *Salmonella spp*

Brilliance *Salmonella* agar base (CM1092) was prepared according to the manufacturer's instruction. One vial of Brilliance *Salmonella* selective supplement (SR0194E) was added for the isolation of *Salmonella spp*. Purple and blue colonies were observed and counted with colony counter, indicated the presence of *Salmonella spp*.

2.3 Statistical Analysis

Data from laboratory analysis are expressed using illustrative tables. Results are expressed as mean ± standard deviation of triplicates. Data obtained were analyzed by one-way analysis of variance (ANOVA) and correlation analysis. Least significance difference (LSD) test was used for means separation for statistical significance at 95% (P<0.05) confidence level, using the statistical software SPSS 17.0 for Windows (SPSS Inc., Chicago, III., USA).

3. RESULTS AND DISCUSSION

3.1 Results

Table 1 shows the bacterial load of water sample (habitat) from MOUAU and Umugbalu locations. Water sample from Umugbalu had the highest TBC of 1.90 x 10^6cfuml^{-1}. Among other pathogenic bacteria, *Coliform* proves to be the most predominating bacteria with the highest value of 1.30 x 10^6cfuml^{-1} from Umugbalu water sample. The next prevalent pathogenic bacteria were *Salmonella spp,* which had a population of 5.0 x 10^5cfuml^{-1} from Umugbalu water sample. *Listeria spp* was not isolated from all the water samples.

Table 2 shows the physiochemical properties of water samples from different locations/habitats where fish species were harvested. For pH, temperature, salinity and turbidity, water sample from Umugbalu location had the highest values of 7.45, 31.5℃, 4.40ppt and 23.0NTU and 7.40 ml/g; while water sample from MOUAU had the lowest value of 5.45, 30.0℃, 4.10ppt and 20.0NTU. MOUAU sample had higher dissolved oxygen value of 7.60 mg/l than Umugbalu sample with a value of 7.60 mg/l.

Table 1. Bacterial count (cfuml^{-1}) of water samples (habitats) for Catfish from different locations

Isolates (Bacterial species)	Locations	
	MOUAU	Umugbalu
TBC	1.70^{c} x 10^6	1.90^{b} x 10^6
Coliforms	1.20^{c} x 10^6	1.30^{b} x 10^6
Listeria	$<10^{1a}$	$<10^{1a}$
Salmonella	4.0^{b} x 10^6	5.0^{c} x 10^6

Each value represents the mean ±SD of three determinations.
Means in the same column with different superscript are significantly different ($p < 0.05$).
MOUAU= Michael Okpara University of Agriculture, Umudike.

Table 2. Physico-chemical characteristics of water (habitat) from which Catfish (*Clarias gariepinus*) were harvested

Characteristics	Locations	
	MOUAU	Umugbalu
pH	5.45^{b}±0.45	7.45^{c}±0.05
Temperature (℃)	30.0^{a}±0.00	31.5^{a}±0.00
Salinity (ppt)	4.10^{b}±0.00	4.40^{b}±0.07
Turbidity (NTU)	20.0^{b}±1.73	23.0^{b}±2.00
Dissolve oxygen (ml/g)	7.60^{b}±0.40	7.40^{c}±0.40

Each value represents the mean ±SD of three determinations.
Means in the same column with different superscript are significantly different ($p < 0.05$).
MOUAU= Michael Okpara University of Agriculture, Umudike

Tables 3, 4 and 5 show the effect of habitat on depuration of Catfish organs (gill, muscles and gut). The total bacteria count (TBC) for the gills, muscle and gut ranged from 1.1 x 10^5 - 1.60 x 10^6cfug^{-1}, 1.2 x 10^5 – 1.47 x 10^6cfug^{-1} and 6.0 x 10^5 – 1.67 x 10^6cfug^{-1} at 48h and 0h of depuration respectively for both habitat locations. 0h is bacterial load before depuration, but at 48 h of depuration there was no isolation of bacteria in both habitats. The *Coliform* values ranged from 2.7 x 105 – 1.00 x 106cfug-^{1}, 2.7 x 10^5 – 9.0 x 10^5 cfug^{-1} and 3.2 x 10^5 – 9.9 x 10^5 cfug^{-1} (at 36 h and 0 h) from Umugbalu location, but were not isolated at 48 h of depuration from both locations. Similarly, *Salmonella spp* had a value of 1.5 x 10^5 - 4.0 x 10^5cfug^{-1}, 1.2 x 10^5 – 3.6 x 10^5 and 2.4 x 10^5 – 4.8 x 10^5cfug^{-1} (at 24h and 0 h). Finally, *Listeria spp* value ranged from

2.0×10^5cfug^{-1} at 0h of depuration but there was no isolation observed from 12 h – 48 h in the *gills and there* was no isolation of *Listeria spp* before and after depuration at the muscles and gut organs of the fish sample.

For gills: Catfish from Umugbalu had the highest TBC value (1.60×10^6) while lowest value was from MOUAU ($<10^1$) at 36 h. *Listeria* was isolated only from MOUAU habitat at 0 h.

For muscle: There was significant difference between the TBC (muscles) from both habitats at 0 h – 24 h hours of depuration. Similarly, there was significant difference between the *Coliform* (gills) from both habitats at 12 h hours of depuration. No significant difference was observed at 0h, 24 h, 36 h and 48 h of depuration between both habitats. Furthermore, at 0h of depuration the *Salmonella spp* load were significantly different from both habitats. From 24 h – 48 h of depuration there was no significant difference in *Salmonella spp* load from both habitats.

For gut: The Least square difference (LSD) of 0.067 shows that there is no significant difference between the TBC (guts) from both habitats. Furthermore, there was significant difference between the *Coliform* load from both habitats at 0 h – 24 h hours of depuration. No significant difference was observed at 36 h and 48 h of depuration between both habitats. The Least square difference (LSD) of 0.382 shows that there is no significant difference between the *Salmonella spp* load (guts) from both habitats.

Table 3. Effect of habitat on bacterial load (cfu/g) of depurated Catfish gills

Depuration periods (h)	Habitat	TBC	Isolates (*Bacterial Spp*)		
			Coliform	***Listeria***	***Salmonella***
0	MOUAU	$1.52^{b} \times 10^6$	$9.9^{a} \times 10^5$	$2.0^{a} \times 10^5$	$3.5^{ab} \times 10^5$
	UMUGBALU	$1.60^{a} \times 10^6$	$1.00^{b} \times 0^6$	$<10^{1c}$	$4.0^{a} \times 10^5$
12	MOUAU	$1.35^{c} \times 10^6$	$8.0^{b} \times 10^5$	$1.5^{b} \times 10^5$	$3.0^{bc} \times 10^5$
	UMUGBALU	$1.01^{d} \times 10^6$	$4.0^{c} \times 10^5$	$<10^{1c}$	$2.8^{c} \times 10^5$
24	MOUAU	$7.2^{d} \times 10^5$	$3.0^{d} \times 10^5$	$<10^{1c}$	$<10^{1e}$
	UMUGBALU	$8.0^{e} \times 10^5$	$3.2^{d} \times 10^5$	$<10^{1c}$	$1.5^{d} \times 10^5$
36	MOUAU	$<10^{1h}$	$<10^{1e}$	$<10^{1c}$	$<10^{1e}$
	UMUGBALU	$4.0^{f} \times 10^5$	$2.7^{d} \times 10^5$	$<10^{1c}$	$<10^{1e}$
48	MOUAU	$<10^{1h}$	$<10^{1e}$	$<10^{1c}$	$<10^{1e}$
	UMUGBALU	$1.1^{g} \times 10^5$	$<10^{1e}$	$<10^{1c}$	$<10^{1e}$
LSD		0.836	0.540	0.983	0.075

Each value represents the mean of triplicate determination
Means in the same column with different superscript are significantly different ($p<0.05$)
MOUAU = Michael Okpara University of Agriculture Umudike.
TBC = Total Bacteria count

Table 4. Effect of habitat on bacterial load (cfu/g) of depurated Catfish muscles

Depuration periods (h)	Habitat	TBC	Isolates (*Bacterial Spp*)		
			Coliform	***Listeria***	***Salmonella***
0	MOUAU	$1.41^{b} \times 10^6$	$9.0^{a} \times 10^5$	$<10^{1a}$	$2.7^{b} \times 10^5$
	UMUGBALU	$1.47^{a} \times 10^6$	$9.3^{a} \times 0^5$	$<10^{1a}$	$3.6^{a} \times 10^5$
12	MOUAU	$4.47^{d} \times 10^5$	$2.7^{c} \times 10^5$	$<10^{1a}$	$1.2^{c} \times 10^5$
	UMUGBALU	$5.6^{c} \times 10^5$	$3.3^{d} \times 10^5$	$<10^{1a}$	$1.4^{c} \times 10^5$
24	MOUAU	$1.2^{f} \times 10^5$	$<10^{1d}$	$<10^{1a}$	$<10^{1d}$
	UMUGBALU	$2.3^{e} \times 10^5$	$<10^{1d}$	$<10^{1a}$	$<10^{1d}$
36	MOUAU	$<10^{1g}$	$<10^{1d}$	$<10^{1a}$	$<10^{1d}$
	UMUGBALU	$<10^{1g}$	$<10^{1d}$	$<10^{1a}$	$<10^{1d}$
48	MOUAU	$<10^{1g}$	$<10^{1d}$	$<10^{1a}$	$<10^{1d}$
	UMUGBALU	$<10^{1g}$	$<10^{1d}$	$<10^{1a}$	$<10^{1d}$
LSD		0.193	0.152	0.522	0.282

Each value represents the mean of triplicate determination
Means in the same column with different superscript are significantly different ($p<0.05$)
MOUAU = Michael Okpara University of Agriculture Umudike
TBC = Total Bacteria Count

Table 5. Effect of habitat on bacterial load (cfu/g) of depurated Catfish guts

Depuration periods (h)	Habitat	TBC	Isolates (*Bacterial Spp)*		
			Coliform	***Listeria***	***Salmonella***
0	MOUAU	$1.60^{a} \times 10^{6}$	$9.5^{a} \times 10^{5}$	$1.5^{b} \times 10^{5}$	$4.0^{b} \times 10^{5}$
	UMUGBALU	$1.67^{a} \times 10^{6}$	$9.9^{a} \times 10^{6}$	$1.8^{a} \times 10^{5}$	$4.8^{a} \times 10^{5}$
12	MOUAU	$1.20^{b} \times 10^{6}$	$7.0^{d} \times 10^{5}$	$<10^{1c}$	$2.6^{c} \times 10^{5}$
	UMUGBALU	$1.15^{b} \times 10^{6}$	$8.5^{d} \times 10^{5}$	$<10^{1c}$	$2.4^{c} \times 10^{5}$
24	MOUAU	$8.0^{c} \times 10^{5}$	$4.5^{e} \times 10^{5}$	$<10^{1c}$	$<10^{1d}$
	UMUGBALU	$6.0^{c} \times 10^{5}$	$3.2^{f} \times 10^{5}$	$<10^{1c}$	$<10^{1d}$
36	MOUAU	$<10^{1d}$	$<10^{1g}$	$<10^{1c}$	$<10^{1d}$
	UMUGBALU	$<10^{1d}$	$<10^{1g}$	$<10^{1c}$	$<10^{1d}$
48	MOUAU	$<10^{1d}$	$<10^{1g}$	$<10^{1c}$	$<10^{1d}$
	UMUGBALU	$<10^{1d}$	$<10^{1g}$	$<10^{1c}$	$<10^{1d}$
LSD		0.067	0.124	0.980	0.382

Each value represents the mean of triplicate determination
Means in the same column with different superscript are significantly different ($p<0.05$).
MOUAU = Michael Okpara University of Agriculture, Umudike.
TBC = Total Bacteria Count.

3.2 Discussion

3.2.1 Bacterial load of water sample (habitat)

The results clearly show substantial difference in the bacteriological condition of water and bacterial load from different fish habitat and parts of fish (the skin, muscle, gills as well as the intestinal tracts. The bacterial load observed in the fish parts was as a result of the bacteria already present in the water where the fishes inhabited. Further impact on bacterial load in water was as a result in the impact of human activities exhibited in the locations. For example, the occurrence of human activities in water inhibited by the fish recorded higher total bacterial count in water (habitat) from Umugbalu which may be attributed to lack of proper sanitary measure by the pond handlers. The feeds used for fish in these ponds may contain organic materials which introduce a wide variety of microorganisms into the ponds [15]. The results of the bacteriological characteristic showed that the selected pathogenic bacteria which were isolated from the water habitat (pond), are gram negative bacteria. However, the occurrence of this gram – negative bacteria in all the locations is an indication of their prevalence in aquatic environment [16,17 and 18]. Additionally, the bacterial variation in different fish species habitat (Table 1) clearly demonstrates the influence of food ecosystem on the bacterial profile of fish in accordance to [19].

The *Coliform* isolated was an indication of the contamination of the pond with fecal material which may result to the presence of pathogenic organism in fish. [15] reported that contamination has been attributed to questionable water quality and high stocking densities.

3.2.2 Physicochemical parameters of water sample (habitat)

The physicochemical parameters of fresh fish species (*Clarias gariepinus*), play a major role in their distribution and microbial profile. Apparently, the temperature obtained (Table 2) is highly favourable for the growth of mesophilic microorganism and that probably explains the high number of TBC, *Coliform* and *Salmonella spp* isolated from the habitat or ecosystem. Furthermore temperature is a factor of great importance for aquaculture ecosystem, as it affects the organism as well as physicochemical properties. Thus, this collaborate with the report of [20], who observed a temperature of 27 – 28℃ in the preliminary studies on water characteristic and bacterial population in Kojalo fish pond. The pH obtained in this study was similar to that of [18], who studied the physicochemical parameters of fish pond water in Okada in Edo state. Also, the pH value of the habitat must have enhanced the growth of the microorganism in both fish species. A pH range of 7.5 to 8.7 has been reported to enhance the survival of fresh fish species [21].

Furthermore, salinity was observed as a factor in the interaction and competitiveness among microorganisms isolated from aquatic environment [17]. Salinity level of 4.10ppt - 6.50ppt in all locations could be as a result of lack of change of the water in aquaculture pond

at appropriate time (handling), which invariably must have brought the water salinity to a moderate level [22]. Salinity is also a major driving factor that affects the density and growth of aquatic organisms' population like freshwater fish [23].

Dissolved oxygen is an important water quality parameter that determines the dynamics of the biota in natural waters because it is a regulator of metabolic processes [24]. Similarly, dissolve oxygen obtained in this study was above 5 mg/l required for fish production. Generally, concentration below 5 mg/l may adversely affect the functions and survival of biological organism and below 3 mg/l can lead to death of most fishes [25]. Low dissolved oxygen observed in a fish pond (water habitat) could be attributed to elevated temperature, increased microbial and organic load and the resultant increase in metabolic activity may also account to low dissolved oxygen concentration [6].

3.2.3 Effect of habitat (pond) on depuration of catfish

The observation of pathogenic bacteria in the organs confirms the finding of [26] that bacteria may be found on the skin, muscle, gills as well as the intestinal tracts of fish or shellfish. However, consumption of fish may cause diseases due to infection or intoxication. Some of these diseases have been specifically associated with pathogens which are resistant to antibiotics and are organisms of public health concern [27]. Furthermore, the micro floras in the fish organ of sea foods such as finfish to some extent are believed to be a reflection of general contamination in the aquatic environment [28]. Therefore, precaution should be taken to prevent water contamination during harvesting as well as post harvest handling of fish. Fish of good quality should have bacterial count less than 10^5 per gram [29] and this was observed from the TBC in different organs. The examined, exceeded acceptable limit recommended by [30]. Nevertheless the bacteria load of other specific pathogenic bacteria (*Coliform*, *Salmonella spp, Listeria spp)* analyzed were not within the acceptable limit less than 10^5 per gram.

Generally the gut of the catfish had a higher bacterial load than gills and muscle; and the reduction rate of bacteria was higher in muscle followed by gills and finally gut. This is in agreement with [28] findings that fish takes a large number of bacteria into their gut from water sediment and food. It is well known that both fresh and brackish water fishes can harbor human pathogenic bacteria particularly the *Coliform* group. Faecal *Coliform* in fish demonstrates the level of pollution in their environment because *Coliform* are not named flora of bacteria in fish [28].

The presence of bacterial load of $2.0 \times 10^5 cfug^{-1}$ *Listeria spp* in the gill of raw freshwater fish material is in agreement with the findings of [18], that up to 96% of the *L. monocytogenes* positive samples were in the gill. Only 4% of the *L. monocytogenes* positive samples were in skin or viscera samples. Freshwater fish (catfish) was contaminated by *L. monocytogenes which* are almost exclusive in the gills and only sporadical in the skin and viscera. On the basis of these results special effort should focus on the isolation and removal of freshwater fish gills before the *L. monocytogenes* contamination may spread further.

The reduction rate of *Coliform* was higher than other bacterial spp. Furthermore, *Coliform* had a higher bacterial count than *Salmonella* and *Listeria spp. Listeria spp was* only isolated from gill before depuration but was not isolated during depuration from all organs. This proves that the penetration ability of microorganisms differ from one organ to the other. [31] reported that the microbial quality of fresh fish indicated that all tissue samples except muscle tissues were contaminated with fecal *Coliform*, where *Escherichia coli* was the most common contaminant and is often encountered in high numbers. Nevertheless, the entire organs had a bacterial count that is lower than that of their habitat and [28] observed that the bacterial load of any fish sample was dependent on the habitat (water) from where they are reared.

The study finally showed that bacterial populations accumulated in freshwater fish (Catfish) were generally reduced to detected levels after depuration for 48h. There was presence of pathogenic bacteria, in the fresh fish water as well as the pond water investigated. This is likely to pose high health risk to humans who use the fresh water fish as source of protein. If it becomes necessary to use fresh water fish as source of protein, then it should be depurated.

4. CONCLUSION

In conclusion, small scale depuration system was adequate for the assessment of bacterial quality

of the water and the freshwater fish (*Clarias gariepinus*) organs. Furthermore, the study also reveals that the water sample (habitat/pond) was grossly contaminated with pathogenic bacteria and the bacteria could affect fish cultivation. These organisms could lower fish yield, cause disease, economic loss and equally endanger the ultimate consumers (humans), particularly if the fish harvested from the water (habitat/pond) are not properly processed. Finally, the TBC and other pathogenic bacteria analyzed initially were not within the acceptable limit. But following depuration for 48 h, the fish organs bacterial load were brought down to a safe level of $<10^5$per gram.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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